

FIG. 1

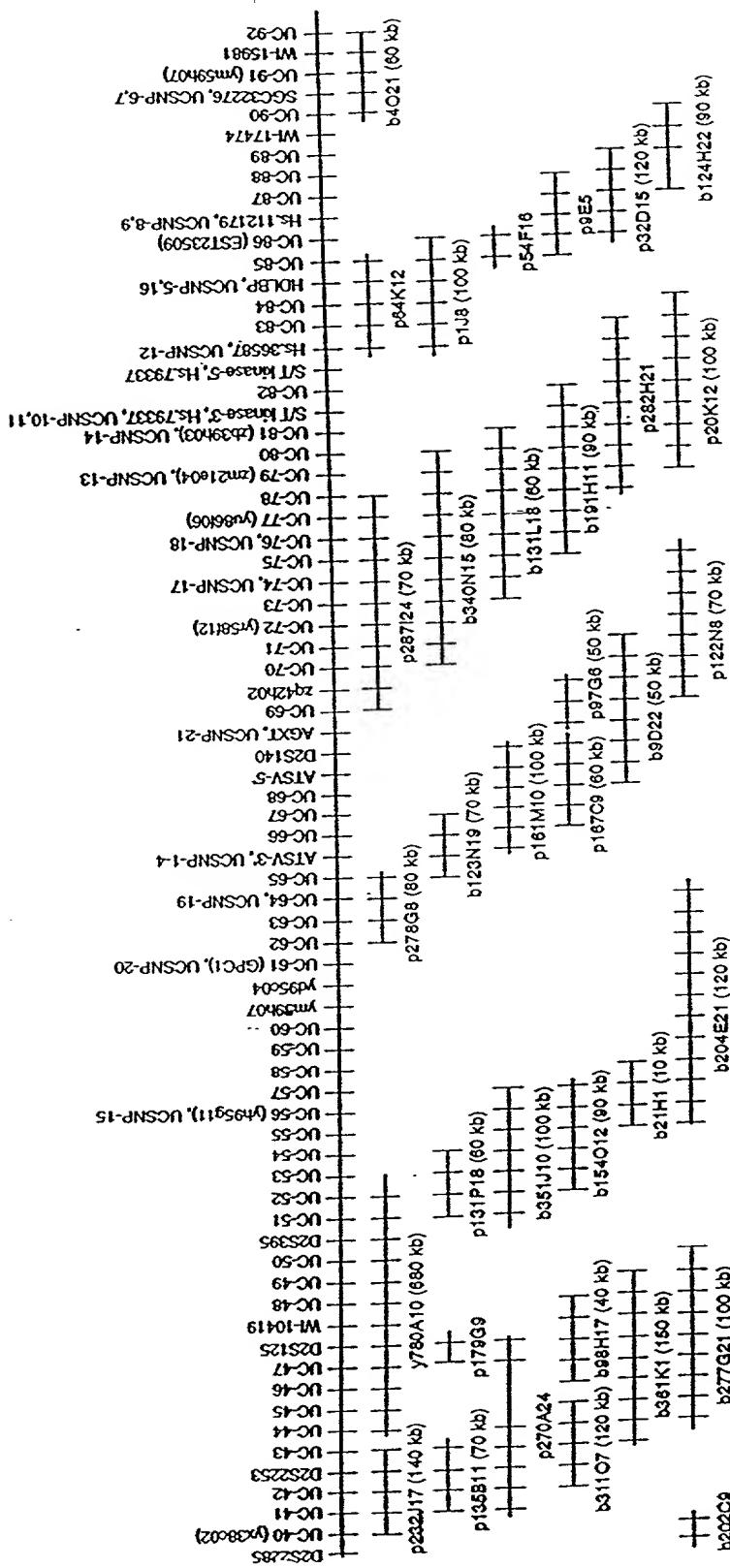
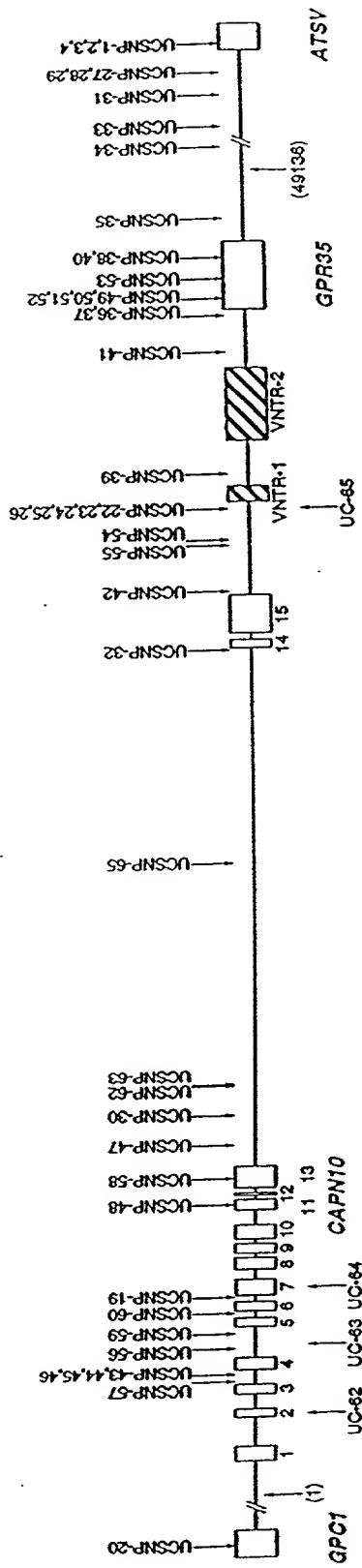


FIG. 2

FIG. 3



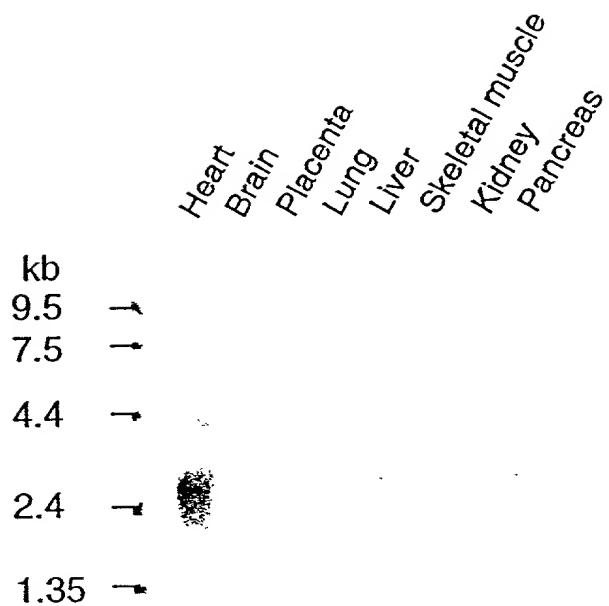


FIG. 4

Domain I ← → Domain II

| | | | |
|---------|--|--|--------------------------|
| ICAPN5 | ----- | HFSCKVKPYEDQNYSLRQDCRRKVLFEDPLFPATDOSLIVY-KGTPGP----- | AVRMKRPKGTCEDPRLEVVDG 67 |
| ICAPN6 | ----- | HCPFLAKLFRNQRYQELQECRKDRGLPCDPFLPENDSLFFNRLLPG----- | KUWKRKPQDISDOPHLIVGN 67 |
| ICAPN3 | MFTVISASVAPRTAAEPRSCPPVPHPAQSKATEAGCGNPSPGIYSALISRNRFIIGVEKETEQEIKKCLEKVKLYVDPPEFPDCTSLFYQKFP----- | IQEVMKRKEPEICENPRFIIDG 215 | |
| ICAPN9 | HYLY----- | KDARITHSSQSSEDFKMRDCEIQRGLTFLFEDADFPASNSLEFSERPQ----- | IPVMKRPGEVILVNPREFLGG 83 |
| ICAPN1 | ----- | RHEAIIKYLQDQYDOLVRCLQSGTFLRDEAFPPVPSLGCKDGMNSKTYGIMKRPTEELSNPQFIVOG 101 | |
| ICAPN2 | ----- | SHERAIKYLQDQYEAELRNCELAEGTFDOPSPFAPLSPALGFKELCPPSSKTRGHMKRPTEICADPOFLGG 91 | |
| ICAPN8 | ----- | SNQKAYKILQDQYETLRKQCLNSGVLFQDPEEPACESALGCKDGMPSOTQEVMKRPTEELCPNPQEIVOG 91 | |
| ICAPN10 | ----- | NRAGRATPARELFKDAAPRADSFLCDSTPLMQRREDITWGRPQEICATPRLEPDD 59 | |

| | | | |
|---------|---|---|--|
| ICAPN5 | ISSHDHIIQGQVNCMEVAACSSLASRESLMQRVIPJMEQE4DPREKACAYAGIFHHFHVRLG-HDVVVIDELRPTVANQLIYCHNSRNEFWCALYKAYAKLACCIQLDGQNTADALV 186 | | |
| ICAPN6 | ISKHOLIQGRLNKAHISAFSCLAVQESHMTRAIPIINHDKDQEWDRPKPEKHFREKIEGEMTEVVVDDOLETTINGDWESFTSHNEWEEAKAYAKLACCIQLDGQNTADALV 187 | | |
| ICAPN3 | ANRTDICOQGLGDCHFLAIACTLTNQHLLFVRF----- | HQSFTEIENYAGIFHFQFQYGEWWDVIVDOLCPLTNNWQWEKTSNHRNEWSALLEKAYAKLACCIQLDGQNTADALV 229 | |
| ICAPN9 | ATRTDICOQGLGDCHFLAIACTLTNQHLLFVRF----- | QDQSPFGPGYAGIFHFQFQHQSKEIQLVYDORLTERDRVLVLSADKNEWSALLEKAYAKLACCIQLDGQNTADALV 197 | |
| ICAPN1 | ATRTDICOQGLGDCHFLAIACTLTNQHLLFVRF----- | HQSFQNYAGIFHFQFQYGEWWDVIVDOLCPLTNNWQWEKTSNHRNEWSALLEKAYAKLACCIQLDGQNTADALV 215 | |
| ICAPN2 | ATRTDICOQGLGDCHFLAIACTLTNQHLLFVRF----- | LQSFQNYAGIFHFQFQYGEWWDVIVDOLCPLTNNWQWEKTSNHRNEWSALLEKAYAKLACCIQLDGQNTADALV 205 | |
| ICAPN8 | ATRTDICOQGLGDCHFLAIACTLTNQHLLFVRF----- | RDSFQNDYAGIFHFQFQYGEWWDVIVDOLCPLTNNWQWEKTSNHRNEWSALLEKAYAKLACCIQLDGQNTADALV 205 | |
| ICAPN10 | PREGQVKQGGLQDCHFLCACAAQKSRILLDQVIP----- | PCQPSWQDYEIRGSFCRIMQFGRWVEVTTDRLCIALGRUCSRCQREDFVMLPLLEKVKWEGSYEHUMAGQVADALV 175 | |

| | | | | |
|---------|---|---|-------------------------|------------------------|
| ICAPN5 | DTFGVVERIDTFLGDFANDETKRNOLFERHLKLVHSRGGLISASIKAV----- | TAADK----- | EARLACGLNKGHAYAVTDV 258 | |
| ICAPN6 | DETGTIAEIIDHOKGRTITDVEEKYFLQFEGTITFKLGICCSIT----- | SQE----- | EVEIDWGLLKGYTHHDI 258 | |
| ICAPN3 | DTFGVVAEFFEIRD----- | AFSDYKIMKKKAIERGSLSHGCSIDUGDTNHTYGTSPSGLNGELIAHVNHNDSNLLQDSDLPRGSDERPTTIIIPVQYETRHAOLVGRHAYSVTG 341 | | |
| ICAPN9 | DTFGVVAETFQTK----- | AENPNTYELLEKALKRNGSLLGCIDT----- | RSAAE----- | EARTFGLLKGHAYSVTG 261 |
| ICAPN1 | DTFGVVAETFLKLK----- | AFSDLYOIJUKALKERGSLLGCSID----- | S----- | EAITFKKLWKGHAYSVTG 279 |
| ICAPN2 | DTFGVVAETFLKLK----- | PPPNLFKTIQKALRKGSLLGCSID----- | S----- | EAITFQKLWKGHAYSVTG 269 |
| ICAPN8 | DTFGGISEFTDLK----- | PFENLYIYDFTDQKALRKGSLLGCSID----- | T----- | EATTROKLWKGHAYSVTG 269 |
| ICAPN10 | DTLGGLAERKMLKGAVSGGQQDPRGRMEHRTCRQIHLHEDQCLISCC----- | VLS----- | PRAGARELGEFHAFIVSDL 245 | |

Domain II ← → Domain III

| | | | | |
|---------|--|--|---|---------------------|
| ICAPN5 | RKVRHLGHGLAFFKSEKLDNIRLNPKGEREENNGPWSDTSEEWQVSKSEREKKGVTVQDGEFWRHTFEDVCRIFTDIIKCRVINTSH-SIHKTWEEARL----- | KGAWTLHEDPRQNIG 373 | | |
| ICAPN6 | RKRLGLERIVEVESTEKLWYRLENPLGRQEWSPMSIIEWEWOQLAVDTRRNGLVHSDOGEFWRHSLEDFCRHNFHKLAVCRVN----- | PVCFKRELESV----- | VGCHTVDDOPLNRS 371 | |
| ICAPN3 | DEVPE----- | KGEKVIWLRNLPQWQVEMNGWSDRWIDWSDVFKDKECARLQRH----- | EDFIVHFTKLEIICNLNTADALQSOKLQHTRIVSVN----- | EGRMWRG-----CSA 444 |
| ICAPN9 | DQVSE----- | RQRIELRNLNPQWQVEMNGWSDSSSEPEWRSVGPQAEQKRLCHTADLGEDEVMAFKOFKAFKDFKWEICNLTPDALEDAIHMKVETVH----- | QCSWRG-----STA 364 | |
| ICAPN1 | KQVNY----- | RQGVVSLIRHMNPWGEVENTCAHMDSSSEPEWRSVGPQAEQKRLCHTADLGEDEVMAFKOFKAFKDFKWEICNLTPDALEDAIHMKVETVH----- | EGTMWRG-----STA 381 | |
| ICAPN2 | EEVES----- | RQSVLQRLRNPQWQVEMNGWSDSSSEPEWRSVGPQAEQKRLCHTADLGEDEVMAFKOFKAFKDFKWEICNLTPDALEDAIHMKVETVH----- | DGMWRG-----STA 371 | |
| ICAPN8 | EEVNE----- | HRGPXKLIRLNLPQWQVEMGSEVNSAMSDNAPEKMYIDPRKEEIDLKAE-DEGEWMSFSDFLQYSRLEICNLTPDITLTSDTYKHLTKH----- | NGRWRG-----STA 371 | |
| ICAPN10 | RELQG----- | QAGCCTILLRIONPQWQRCWQGLMREGCEGHQSVDAAVASELLSQLQ-EGEPWVVEEEDELFREFELDTLVGPTVTEAGHQSLITERLICHTRALPGAKWKG-----QSA 352 | | |

| | | | |
|---------|--------------------------------|--|---|
| ICAPN5 | GCGCINRDTFFQNPQYIFEVVKPED----- | EVLICIQQPRKRSTRRECGKGENLAIGFDIYKVE----- | E-----NRQYHESL-----QKAASSITYINSRSVFLRTDQPEGRYVIPT 472 |
| ICAPN6 | GCGCINRDTFFQNPQYIFEVVKPED----- | KVHNSLQKQDIAITTYRCRDPDNTYIGFLKVE----- | H-----NRERLHLYTQERAGTSTIDYIIRKELVSKLAKGSKYVILVT 472 |
| ICAPN3 | GCCRNPDTWTPQYQLLLEEDD----- | DEPDSEIC-CSFLVALMQLQNRKDRKLKRCG-ASLVTIGAIVPEKEMGNK-OLHODFLFLYNAKSARSKTINYREVRSQRFPLPSEYIVIPS 559 | |
| ICAPN9 | GCCRNFDTWTPQYQLLLEEDD----- | CQEE----- | CSFLVALMQLQNRKDRKLKRCG-ASLVTIGAIVPEKEMGNK-OLHODFLFLYNAKSARSKTINYREVRSQRFPLPSEYIVIPS 471 |
| ICAPN1 | GCCRNPDTWTPQYQLLLEEDD----- | DEPDSEIC-CSFLVALMQLQNRKDRKLKRCG-ASLVTIGAIVPEKEMGNK-OLHODFLFLYNAKSARSKTINYREVRSQRFPLPSEYIVIPS 471 | |
| ICAPN2 | GCCRNPDTWTPQYQLLLEEDD----- | DEPDSEIC-CSFLVALMQLQNRKDRKLKRCG-ASLVTIGAIVPEKEMGNK-OLHODFLFLYNAKSARSKTINYREVRSQRFPLPSEYIVIPS 471 | |
| ICAPN8 | GCCRNPDTWTPQYQLLLEEDD----- | DEPDSEIC-CSFLVALMQLQNRKDRKLKRCG-ASLVTIGAIVPEKEMGNK-OLHODFLFLYNAKSARSKTINYREVRSQRFPLPSEYIVIPS 471 | |
| ICAPN10 | GCCRNPDTWTPQYQLLLEEDD----- | DEPDSEIC-CSFLVALMQLQNRKDRKLKRCG-ASLVTIGAIVPEKEMGNK-OLHODFLFLYNAKSARSKTINYREVRSQRFPLPSEYIVIPS 471 | |

| | | | |
|---------|-------|-------|-------|
| ICAPN5 | ----- | ----- | ----- |
| ICAPN6 | ----- | ----- | ----- |
| ICAPN3 | ----- | ----- | ----- |
| ICAPN9 | ----- | ----- | ----- |
| ICAPN1 | ----- | ----- | ----- |
| ICAPN2 | ----- | ----- | ----- |
| ICAPN8 | ----- | ----- | ----- |
| ICAPN10 | ----- | ----- | ----- |

Domain III ← → Domain IV or V

| | | | |
|---------|--|--------|-----------------------------------|
| ICAPN5 | TTFEPGHTGEFLRVTIDVSNCRELDRDPHT----- | C----- | KSSLCGTYPOLIVQVHVLGAGLKD----- 530 |
| ICAPN6 | TTFEPGHTGEFLRVTIDVSNCRELDRDPHT----- | C----- | WNLARCTPKVTVQIYTSAEGLKKY----- 532 |
| ICAPN3 | TTFEPGHTGEFLRVTSEKRNLESEVENTISVDRPKKKKTRPIIFVSDRANSKELGVQDQESEBGKKTSPDKQKSPQQPQFSSDQESEBQFQKQIAGDKOECIACADELAKVYLN 473 | | |
| ICAPN9 | TTFEPGHTGEFLRVTSEKRNLESEVENTISVDRPKKKKTRPIIFVSDRANSKELGVQDQESEBGKKTSPDKQKSPQQPQFSSDQESEBQFQKQIAGDKOECIACADELAKVYLN 473 | | |
| ICAPN1 | TTFEPGHTGEFLRVTSEKRNLESEVENTISVDRPKKKKTRPIIFVSDRANSKELGVQDQESEBGKKTSPDKQKSPQQPQFSSDQESEBQFQKQIAGDKOECIACADELAKVYLN 473 | | |
| ICAPN2 | TTFEPGHTGEFLRVTSEKRNLESEVENTISVDRPKKKKTRPIIFVSDRANSKELGVQDQESEBGKKTSPDKQKSPQQPQFSSDQESEBQFQKQIAGDKOECIACADELAKVYLN 473 | | |
| ICAPN8 | TTFEPGHTGEFLRVTSEKRNLESEVENTISVDRPKKKKTRPIIFVSDRANSKELGVQDQESEBGKKTSPDKQKSPQQPQFSSDQESEBQFQKQIAGDKOECIACADELAKVYLN 473 | | |
| ICAPN10 | TTFEPGHTGEFLRVTSEKRNLESEVENTISVDRPKKKKTRPIIFVSDRANSKELGVQDQESEBGKKTSPDKQKSPQQPQFSSDQESEBQFQKQIAGDKOECIACADELAKVYLN 473 | | |

| | | | |
|---------|-------|-------|-------|
| ICAPN5 | ----- | ----- | ----- |
| ICAPN6 | ----- | ----- | ----- |
| ICAPN3 | ----- | ----- | ----- |
| ICAPN9 | ----- | ----- | ----- |
| ICAPN1 | ----- | ----- | ----- |
| ICAPN2 | ----- | ----- | ----- |
| ICAPN8 | ----- | ----- | ----- |
| ICAPN10 | ----- | ----- | ----- |

Domain IV or V

| | | | |
|---------|-------|-------|-------|
| ICAPN5 | ----- | ----- | ----- |
| ICAPN6 | ----- | ----- | ----- |
| ICAPN3 | ----- | ----- | ----- |
| ICAPN9 | ----- | ----- | ----- |
| ICAPN1 | ----- | ----- | ----- |
| ICAPN2 | ----- | ----- | ----- |
| ICAPN8 | ----- | ----- | ----- |
| ICAPN10 | ----- | ----- | ----- |

FIG. 5

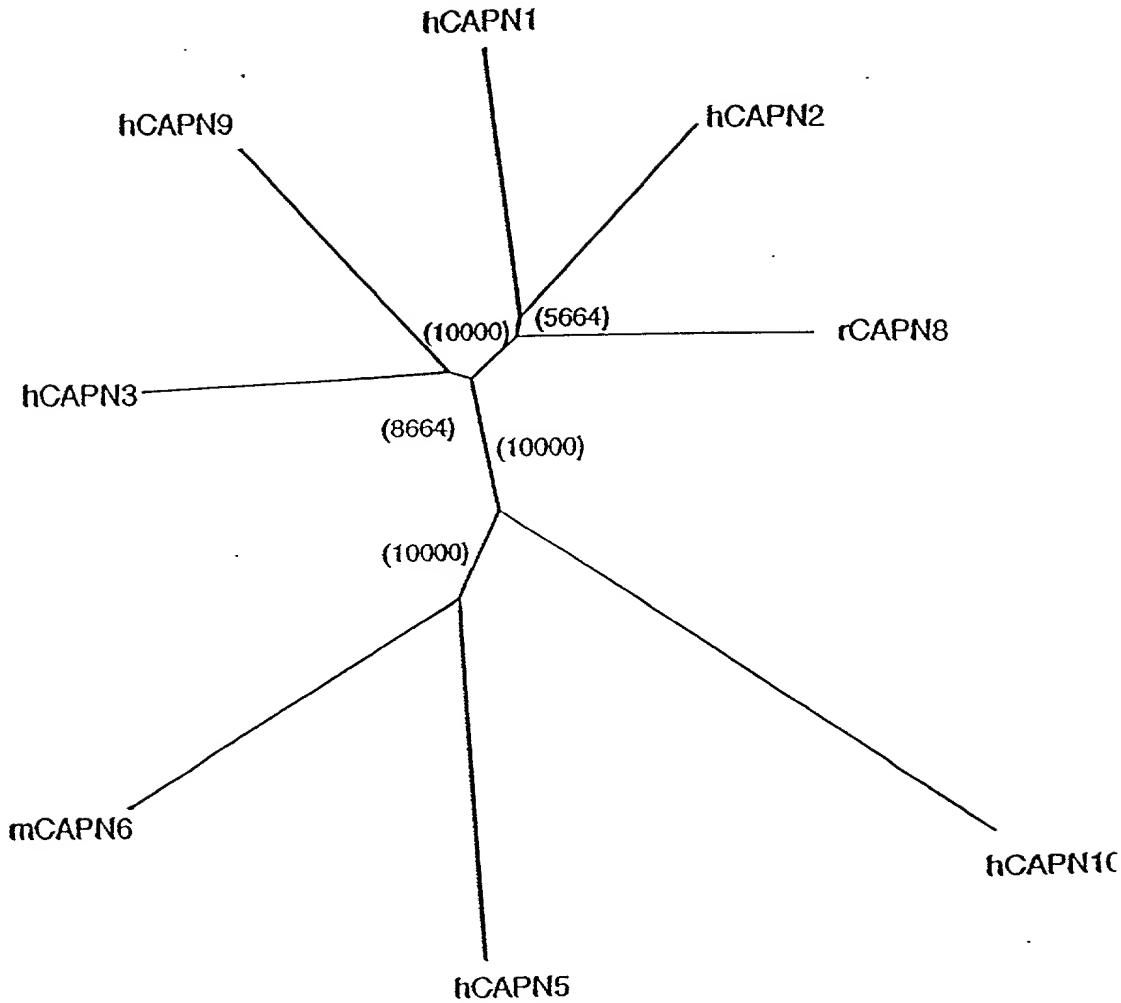
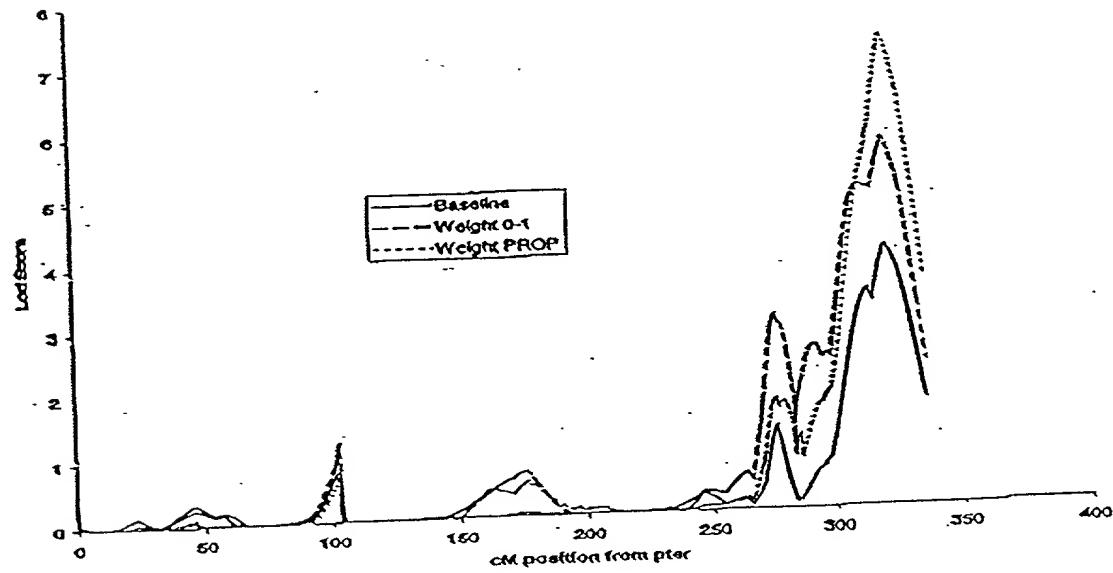
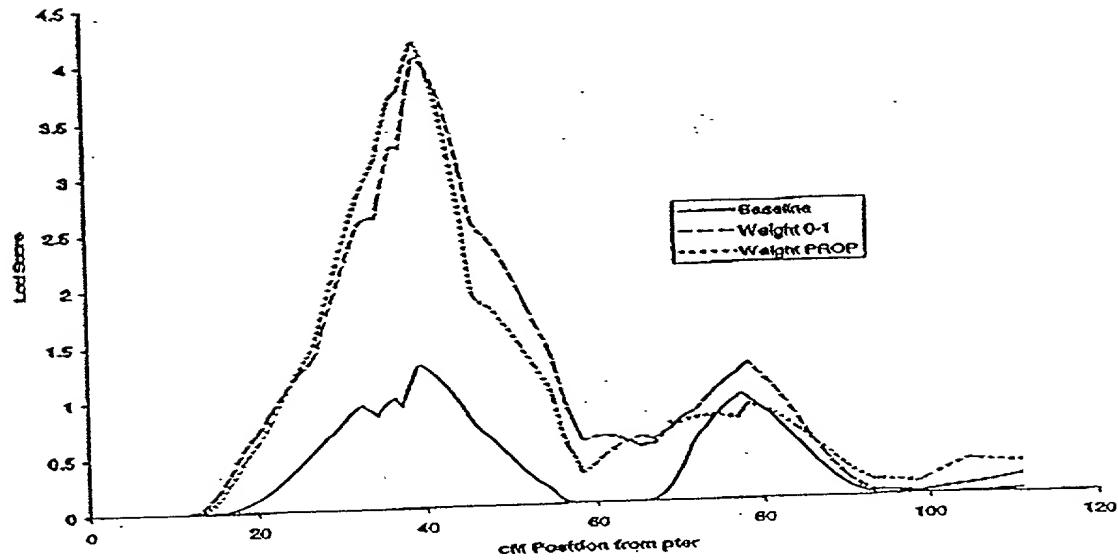


FIG. 6



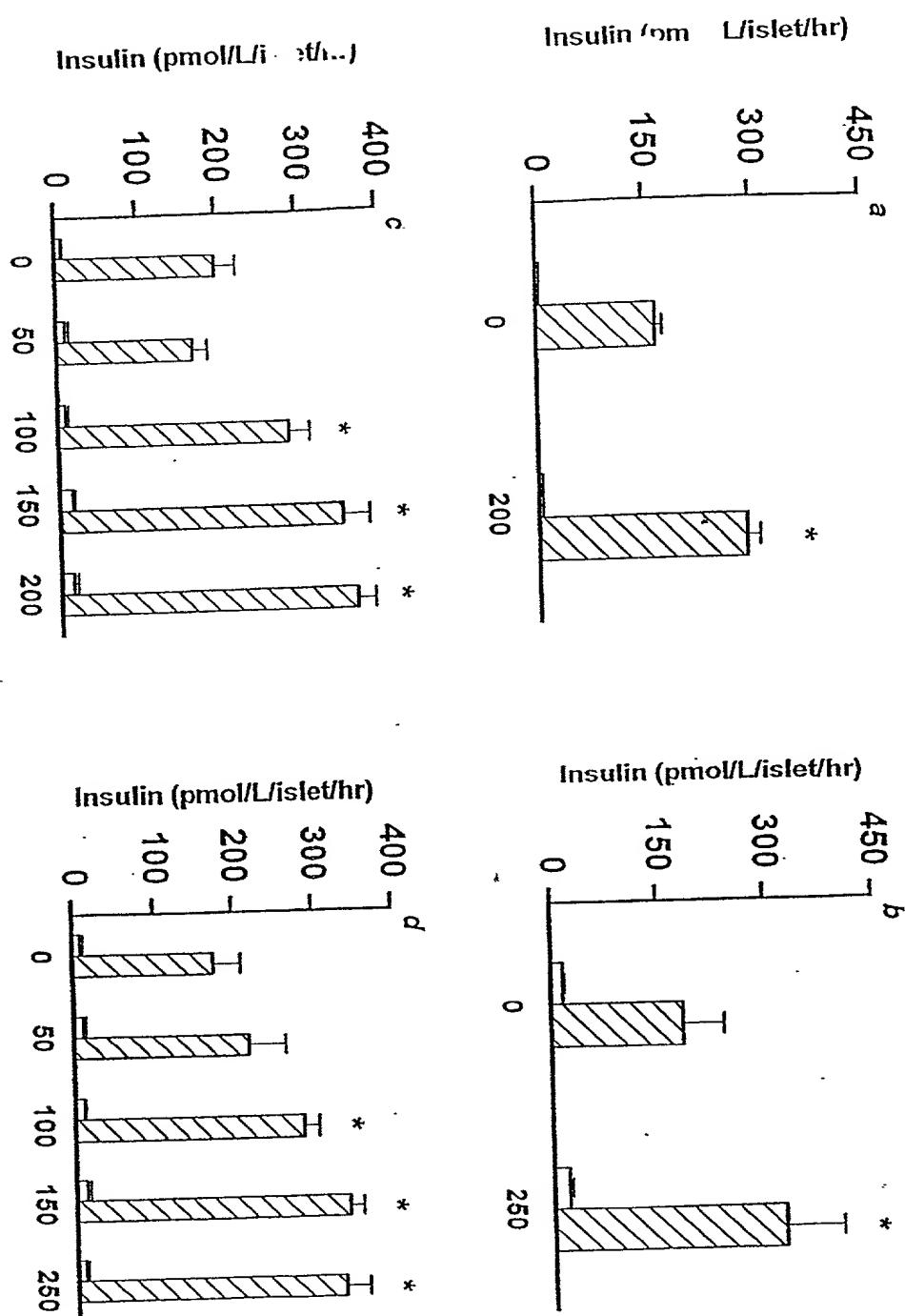


FIG. 8

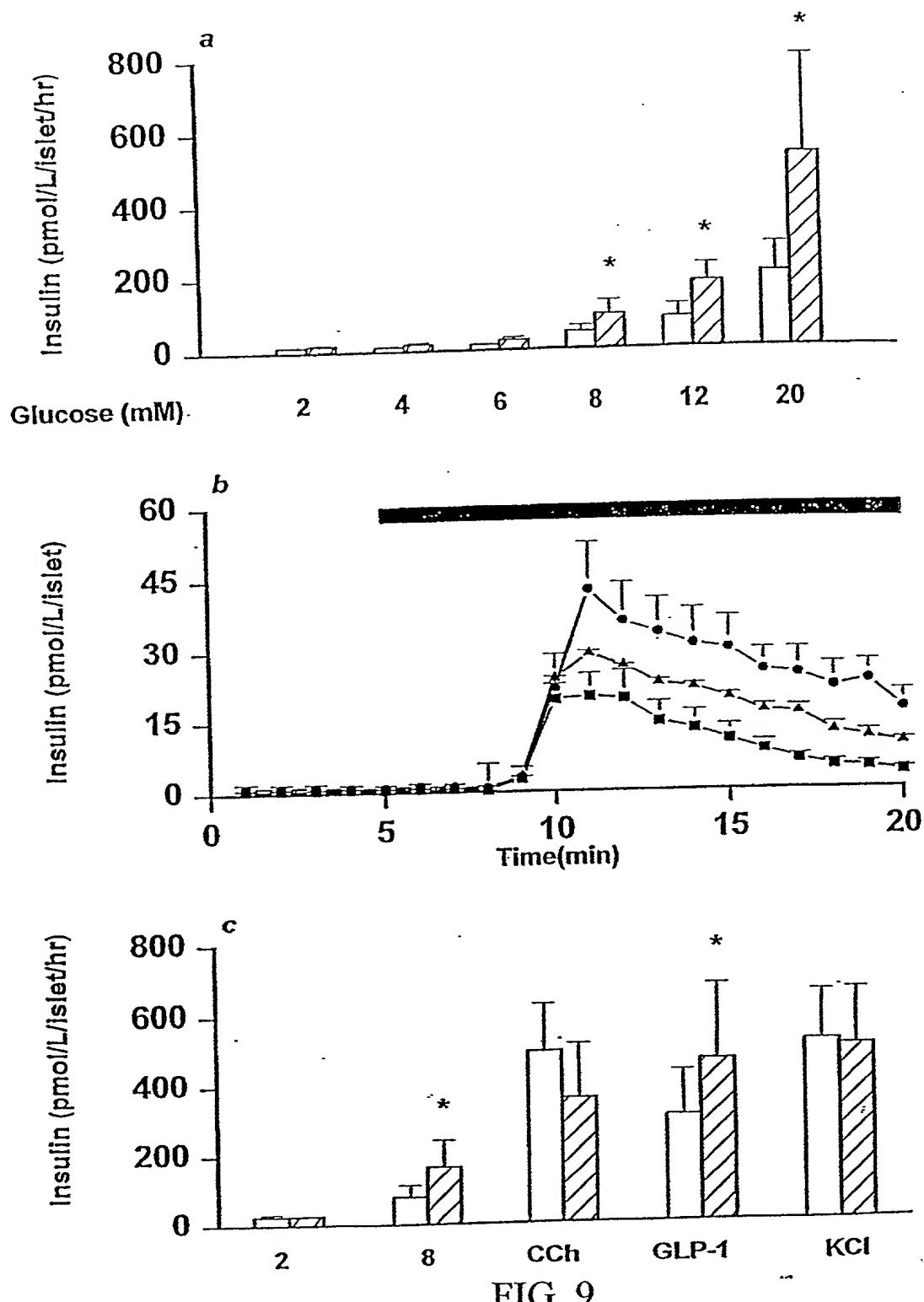
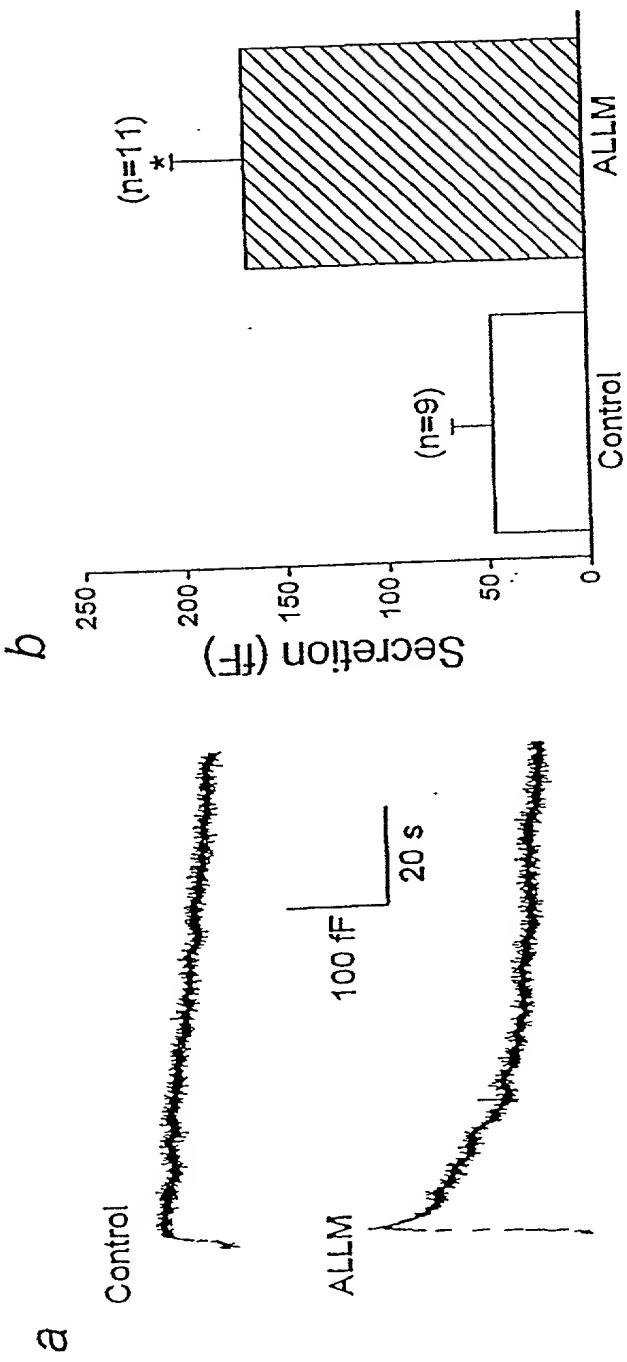


FIG. 9

FIG. 10



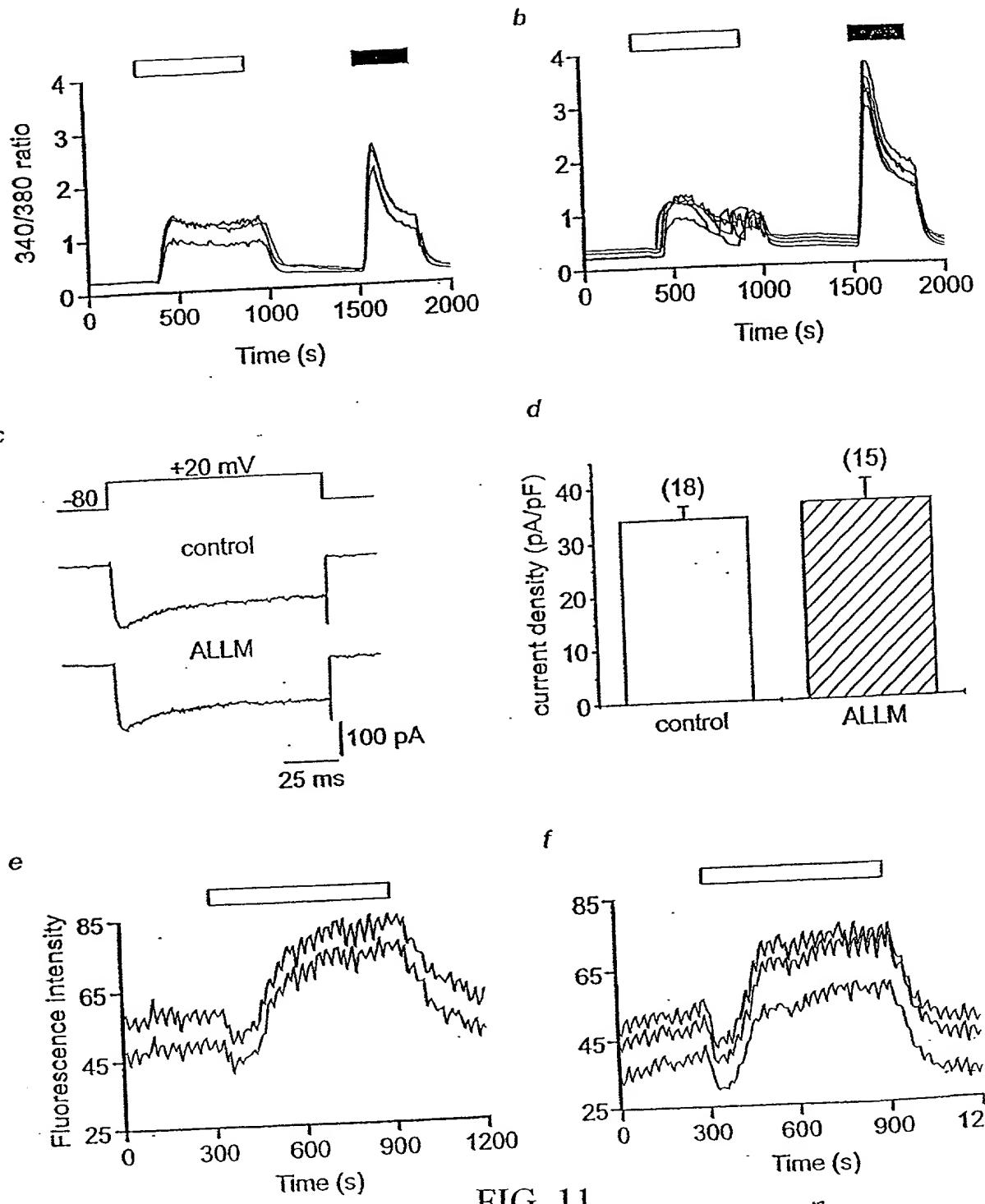


FIG. 11

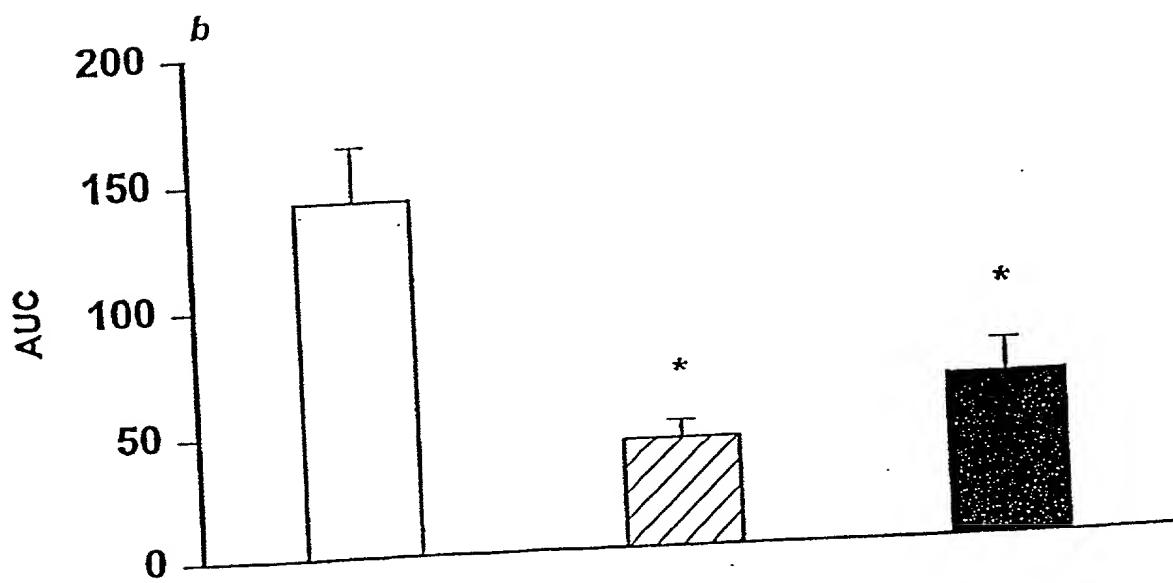
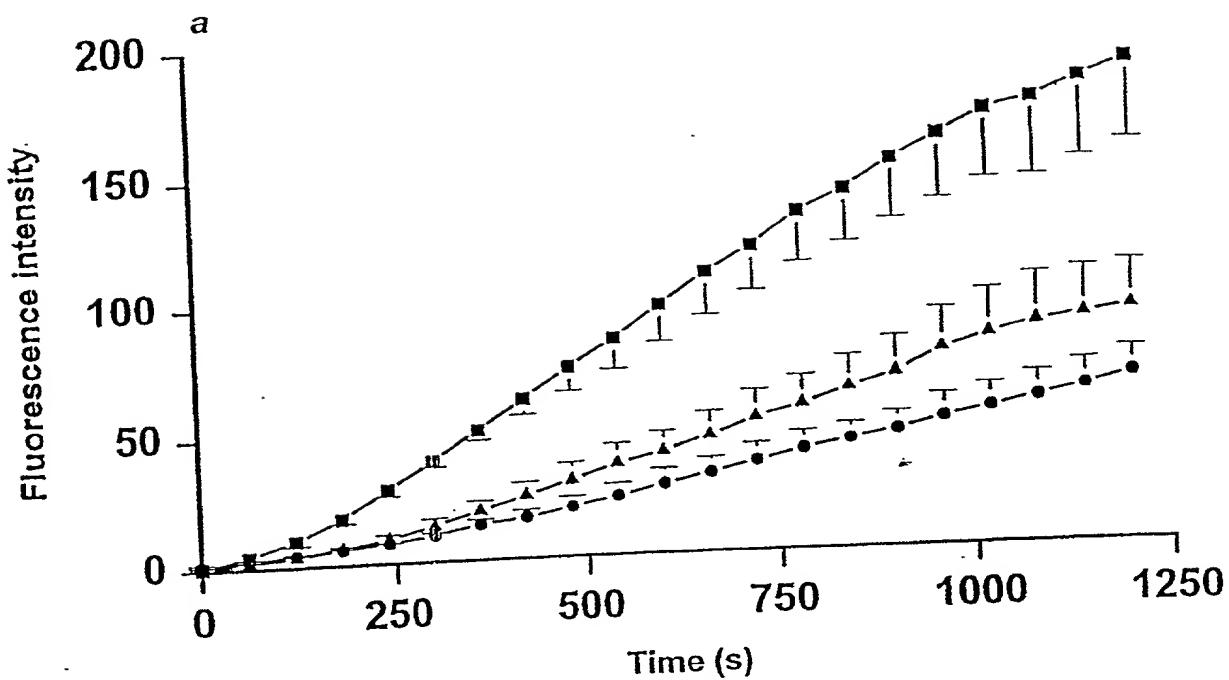


FIG. 12

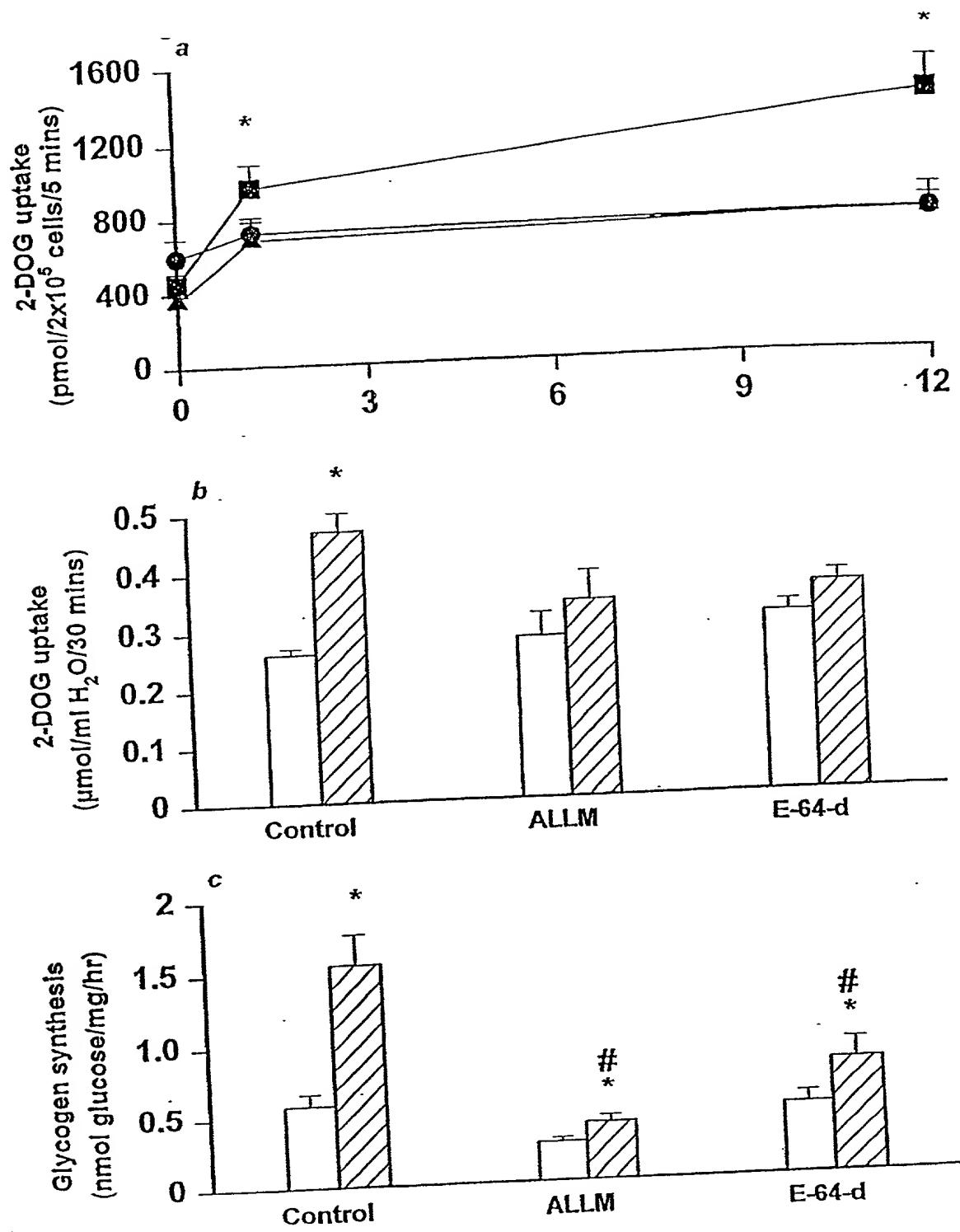


FIG. 13

Fig14. Effect of 48 hours exposure of islets to calpain inhibitors on insulin secretion

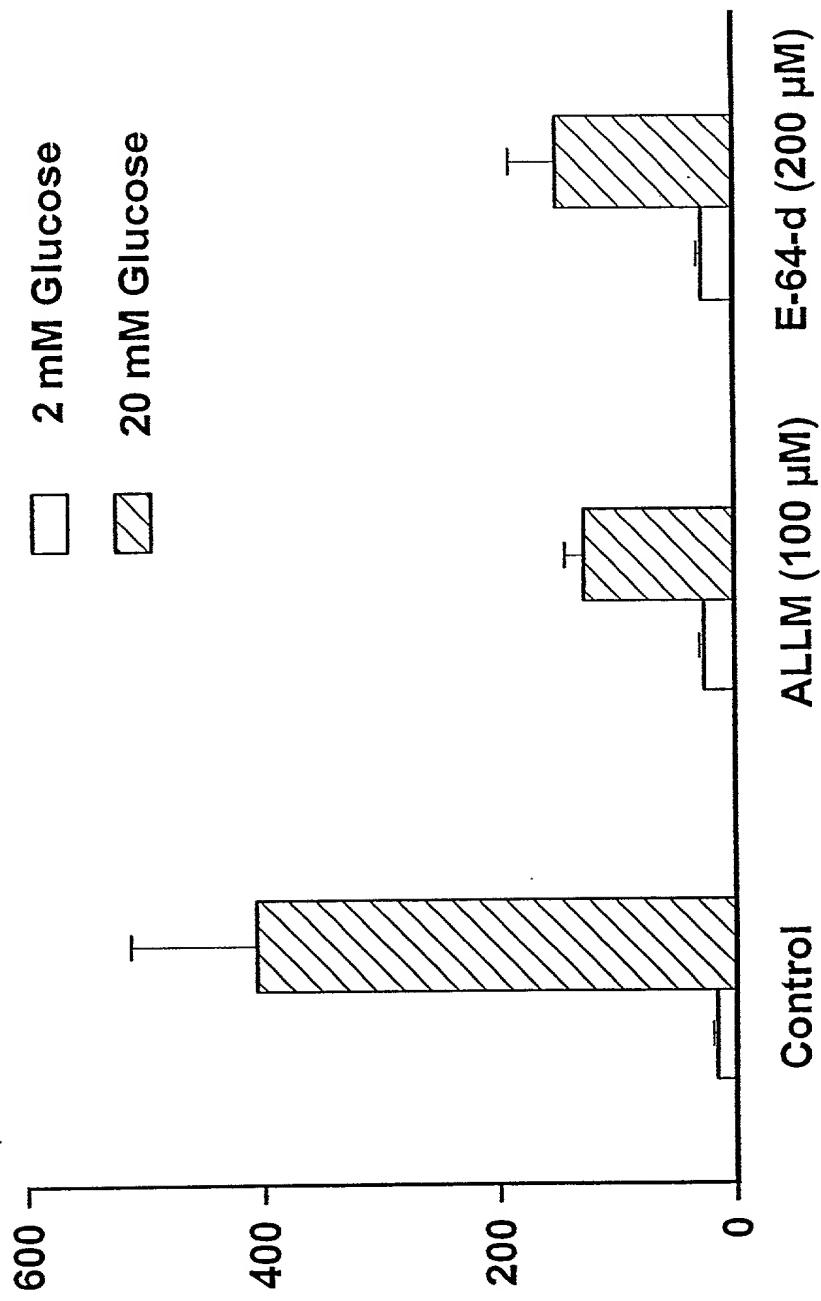


Fig 15 Insulin content in 48 hour cultured islets ($n=4$)

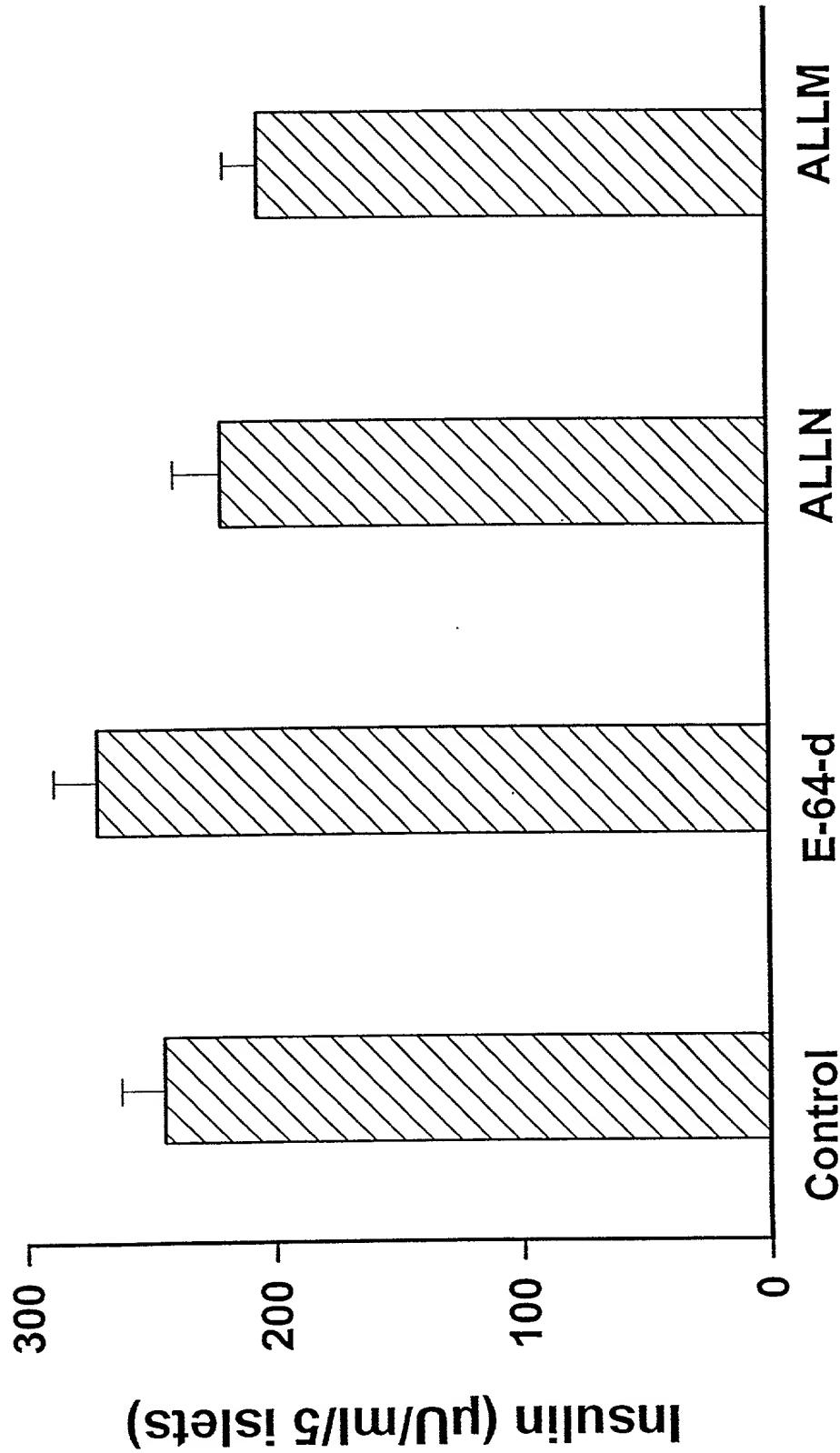


Fig 16. ALLM dose response in 48 hour treated islets

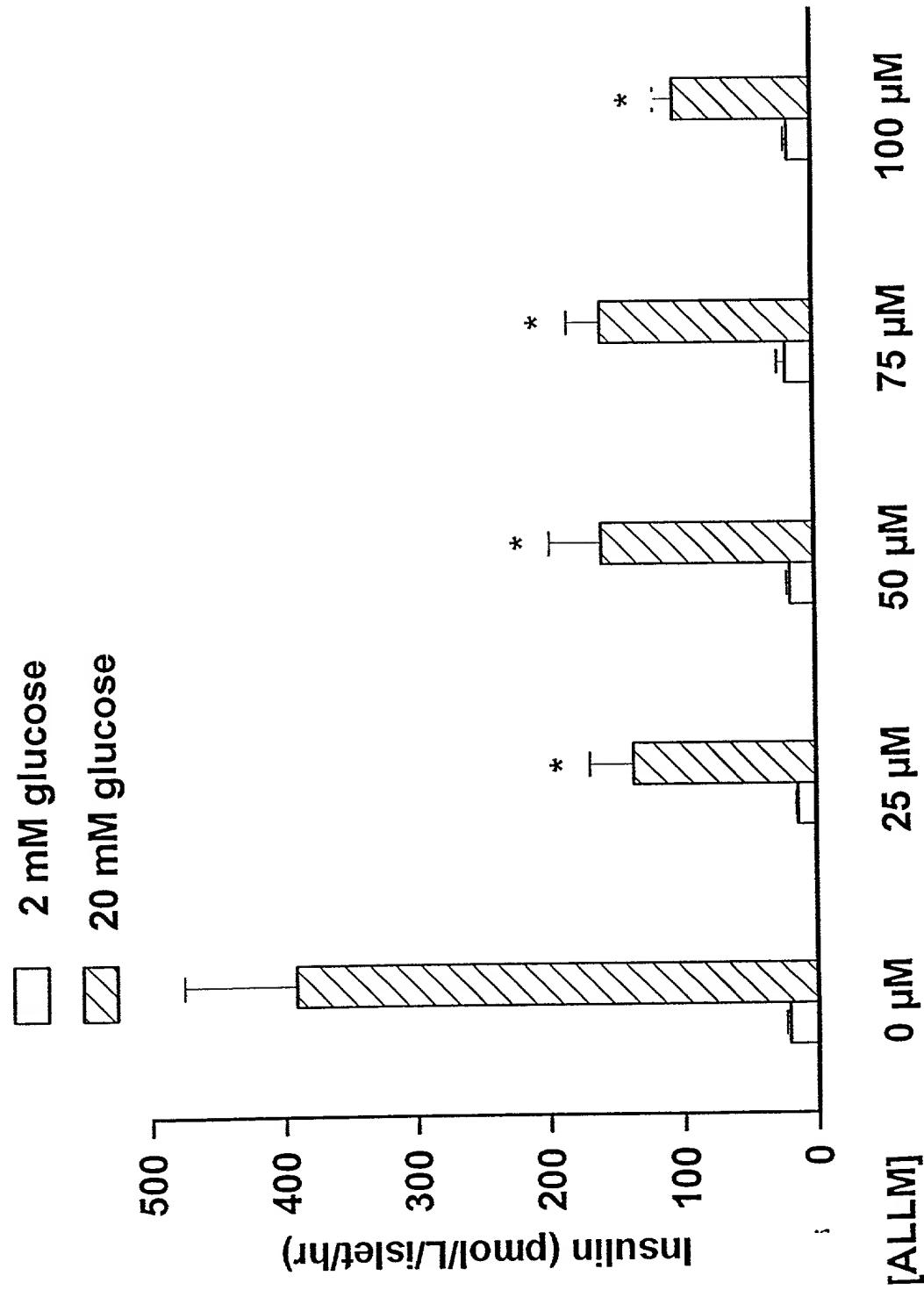
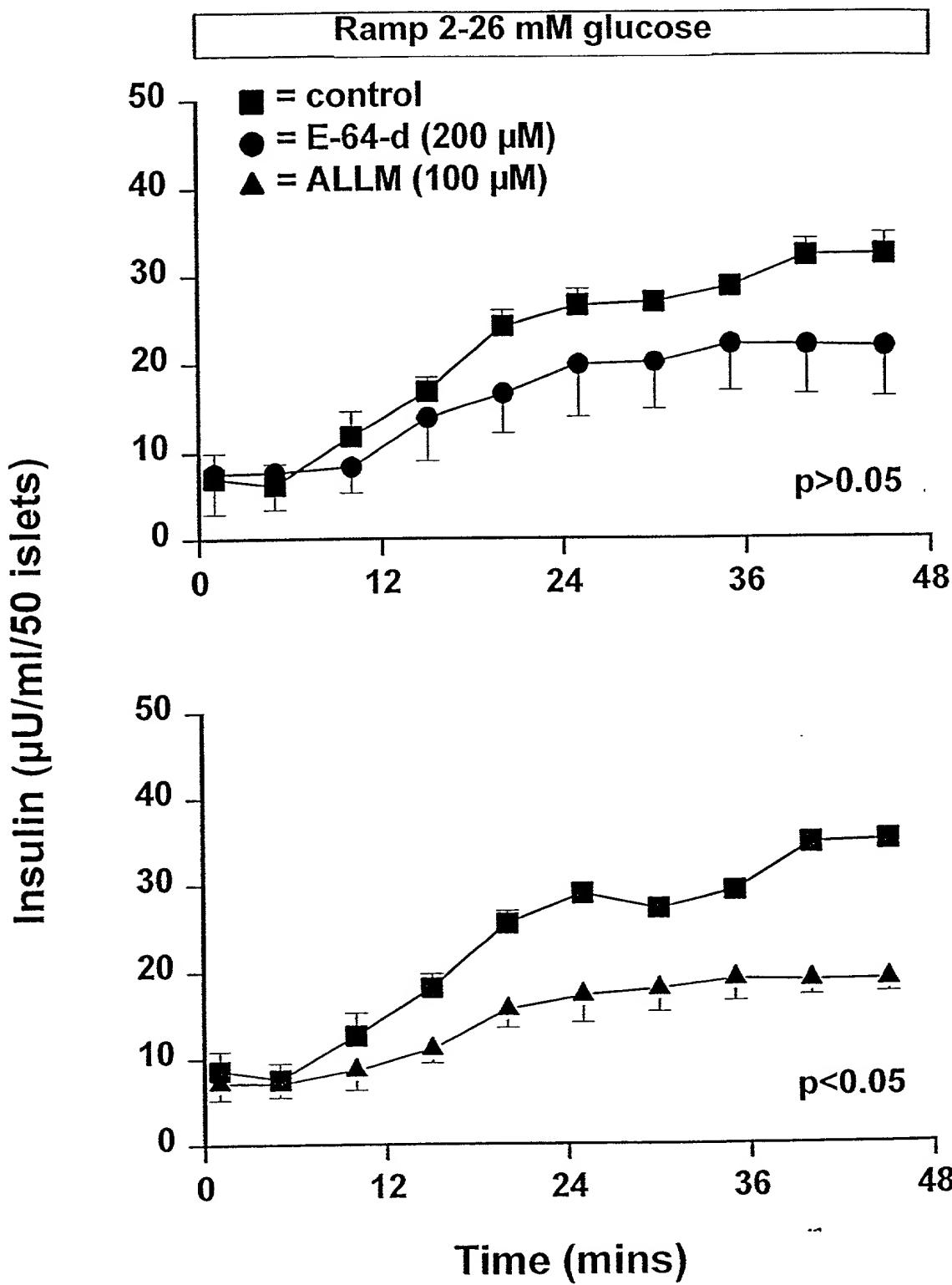


Fig 17. Perifusion of 48 hour cultured islets (n=4)



Treatment of E64treated mice
with ALLM or E64-d

Insulin secretion in ALLM or E64-d treated mouse
islets: Reversal study

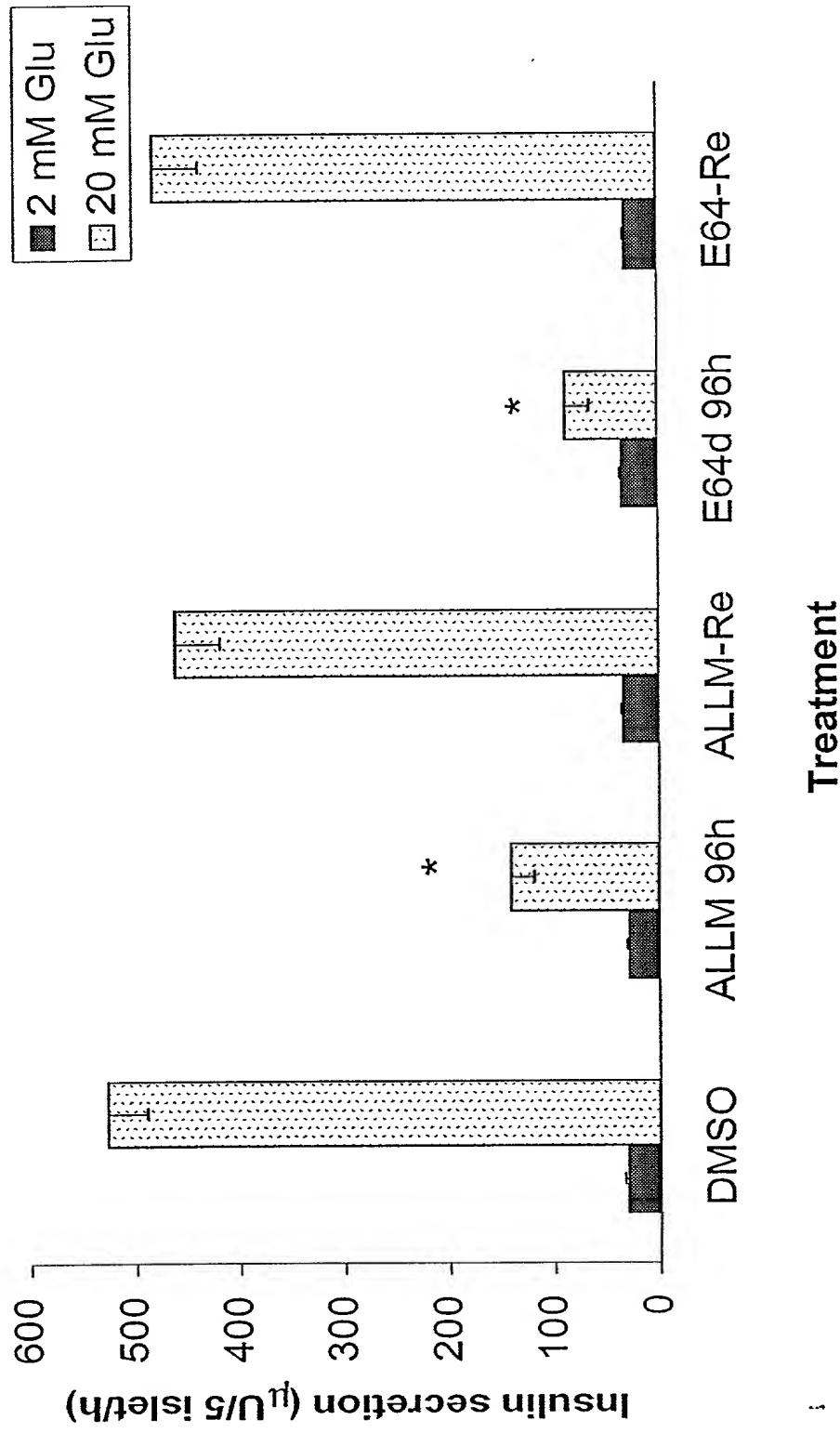
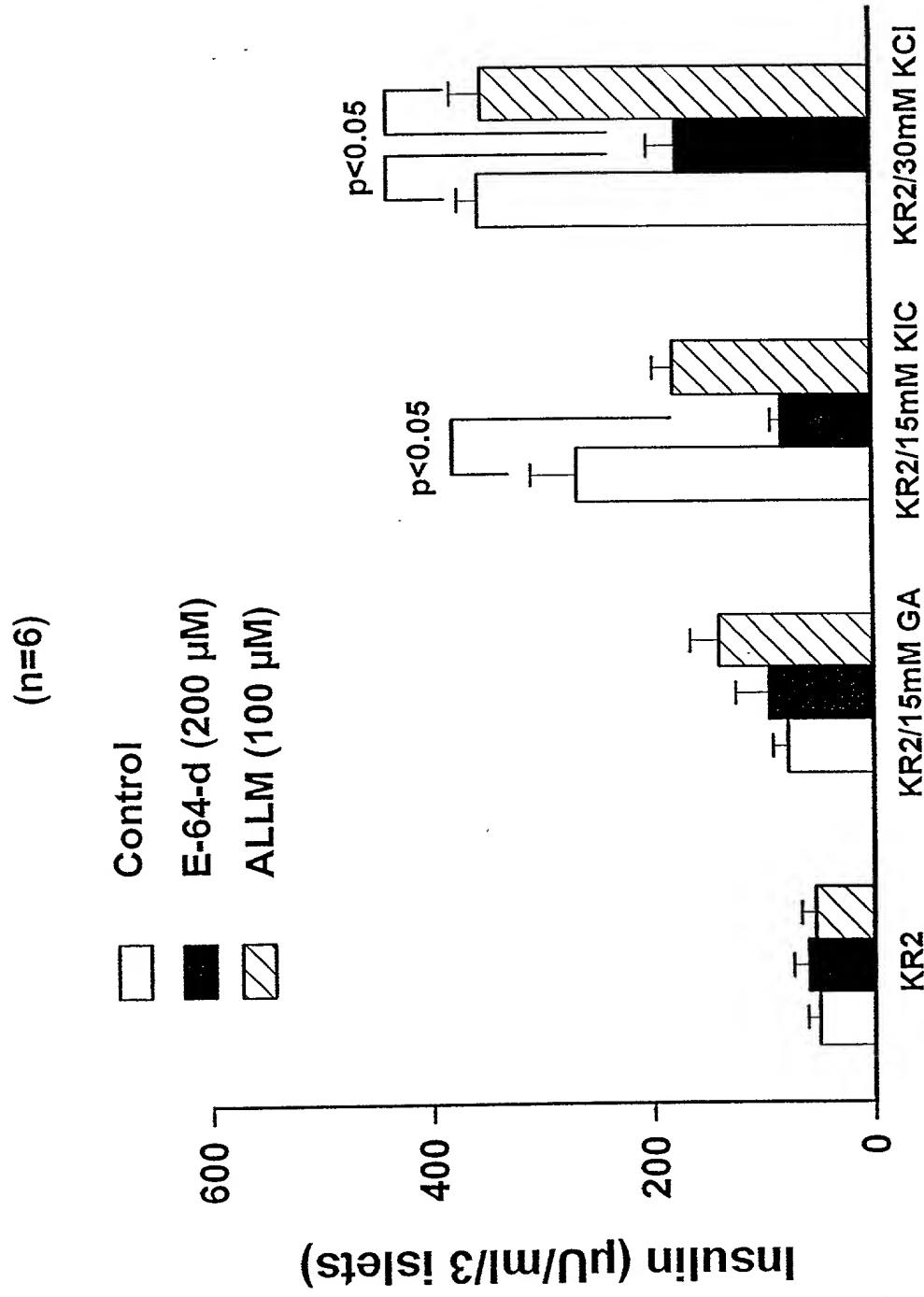


Fig. 18

Fig 19. Insulin secretion by islets following exposure to calpain inhibitors for 48 hrs



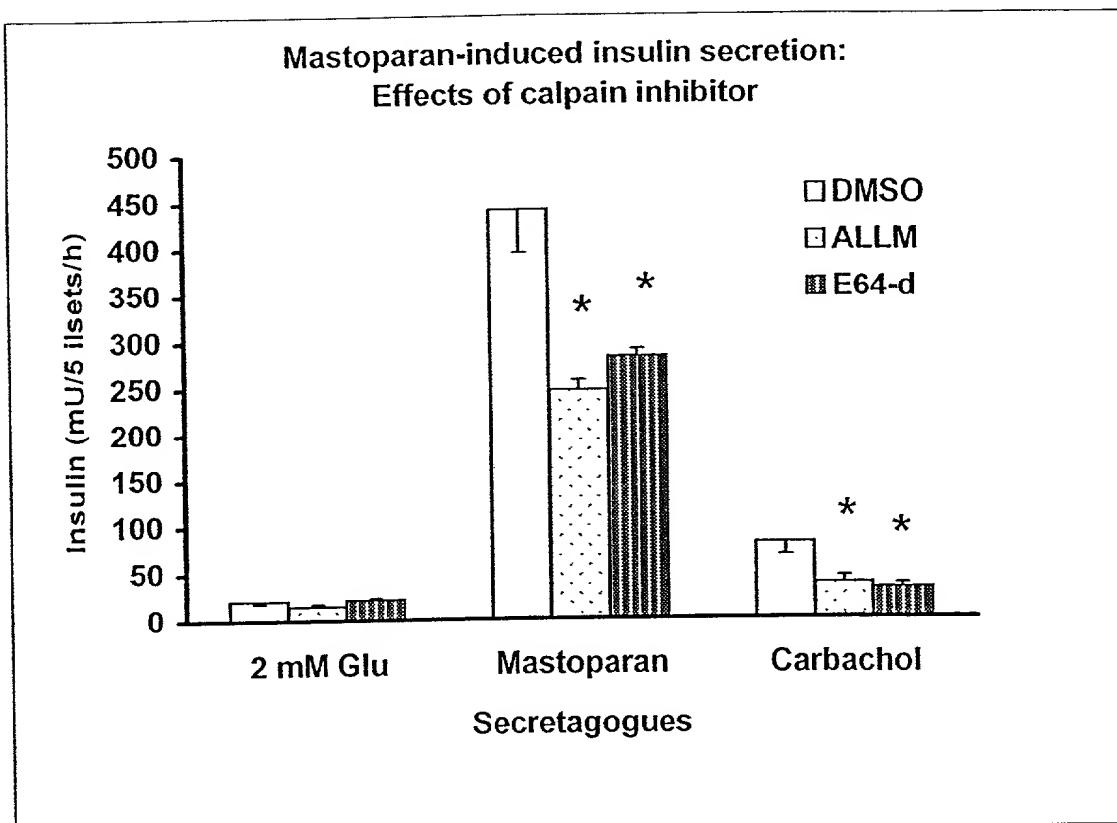
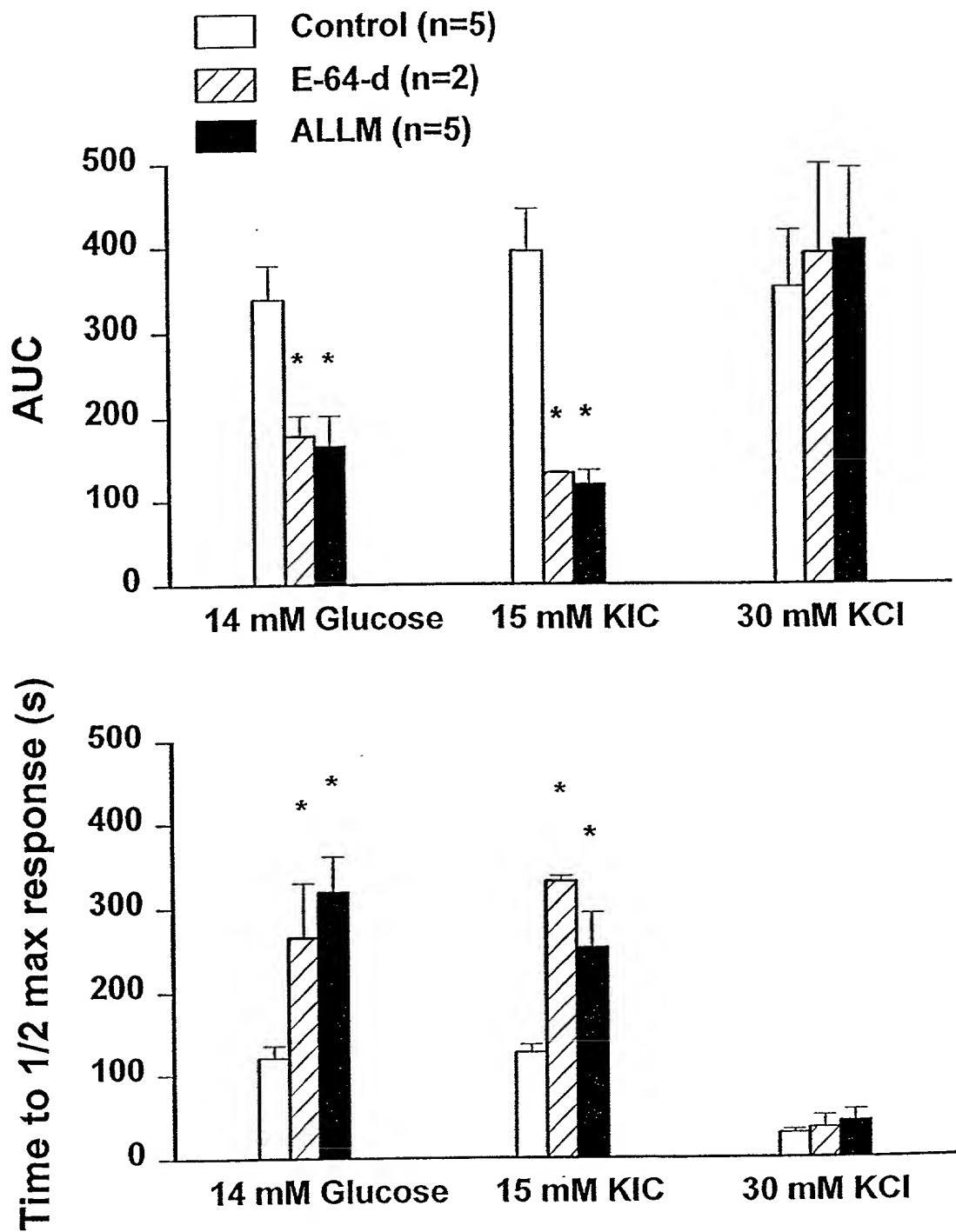


FIG. 2D

Fig 21. $[Ca^{2+}]_i$ responses to glucose, KIC and KCl



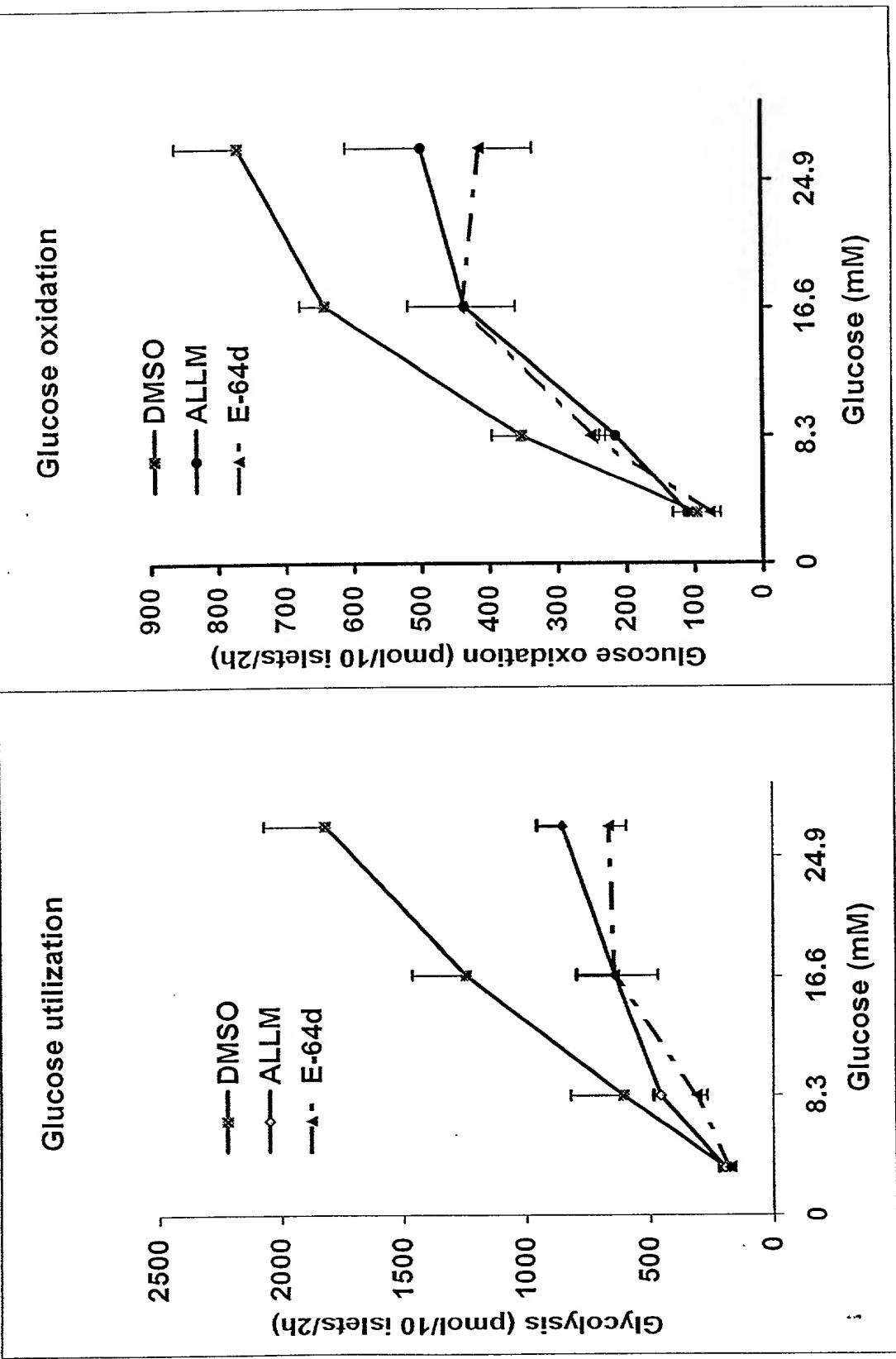
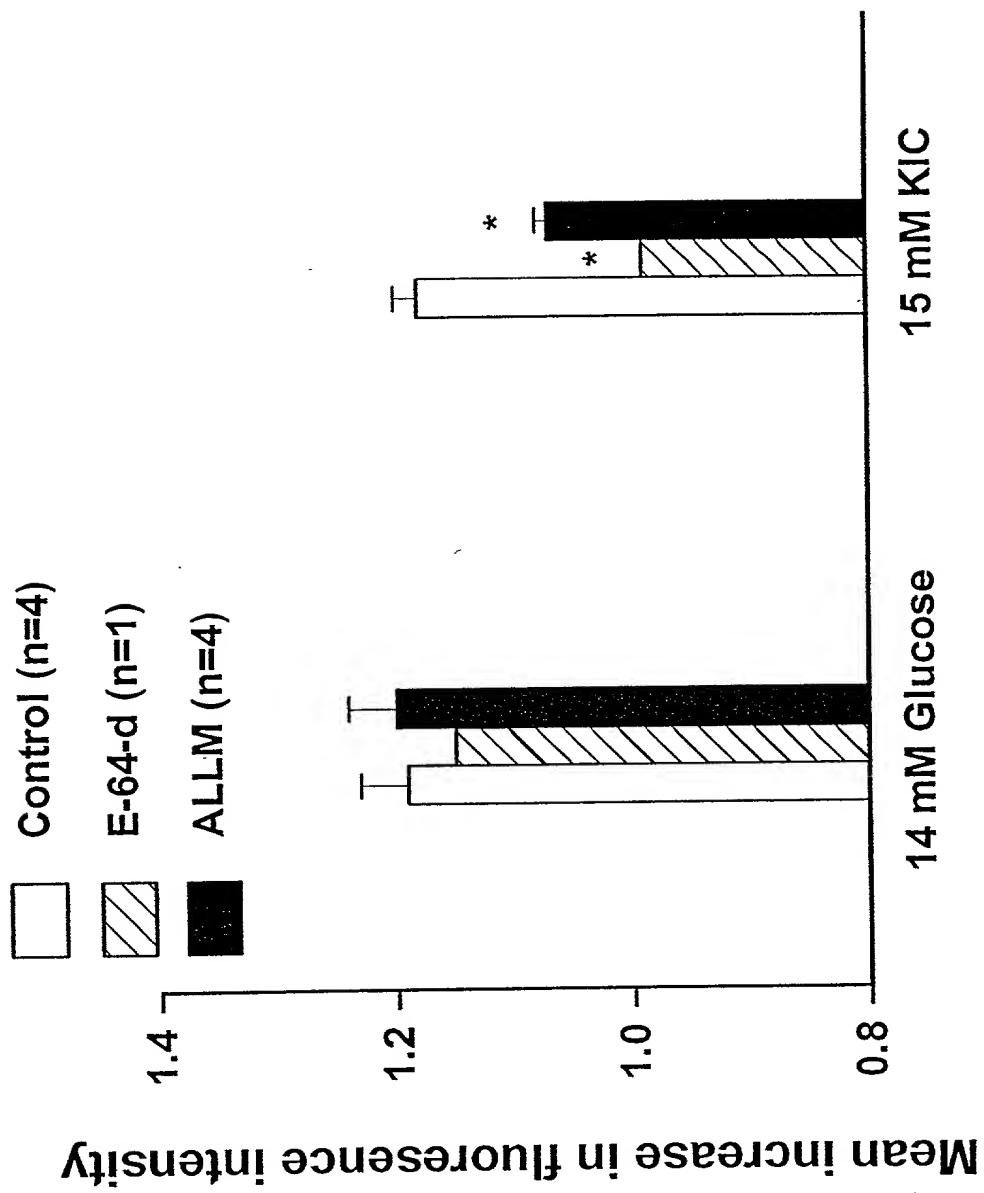


Fig. 22

Fig 23. NAD(P)H responses to glucose and KIC



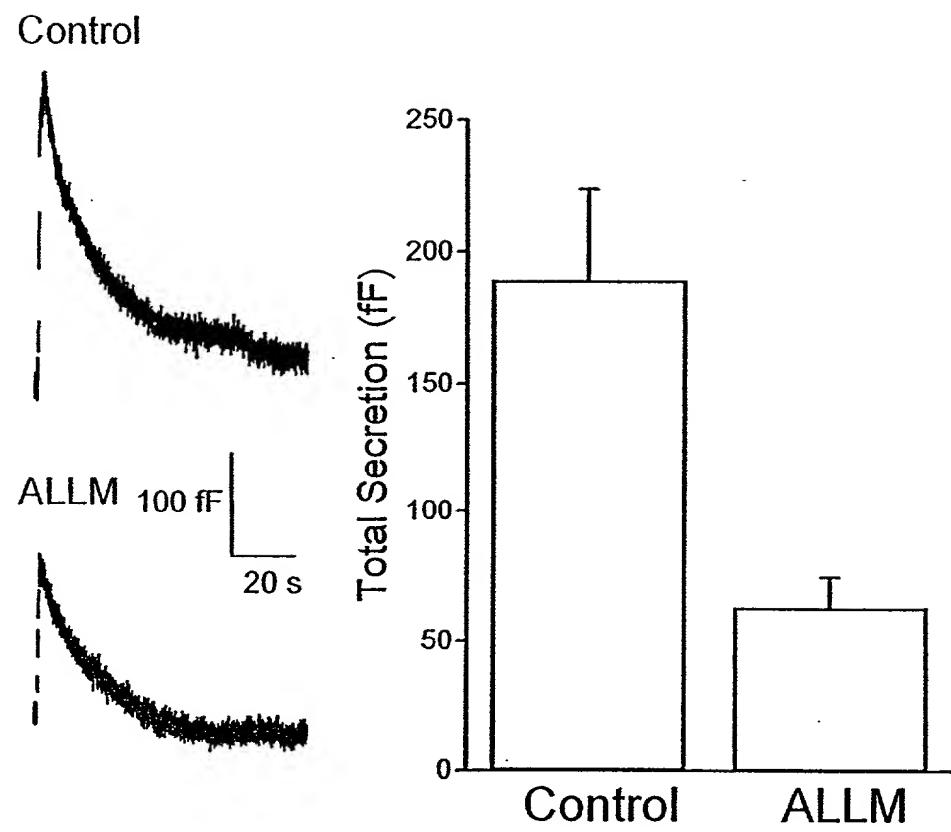
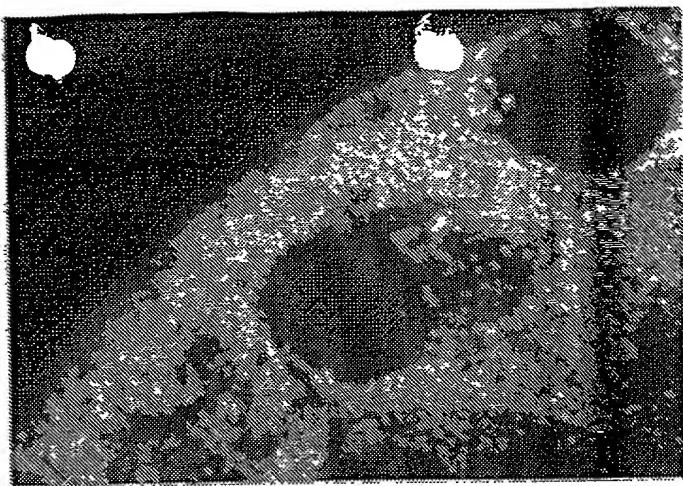
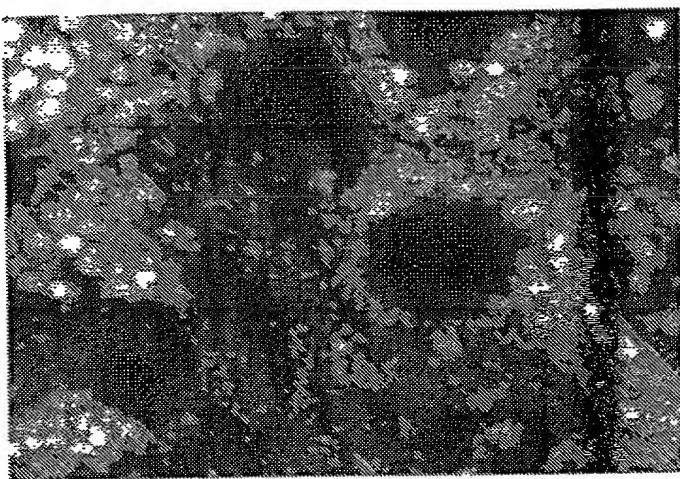


Fig. 24. Measurement of membrane capacitance in isolated β -cells

DMSO



E64d



ALLM

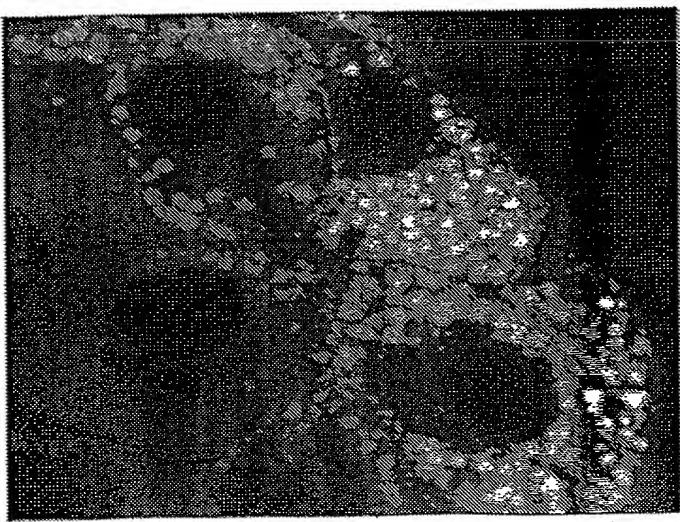


FIG.25

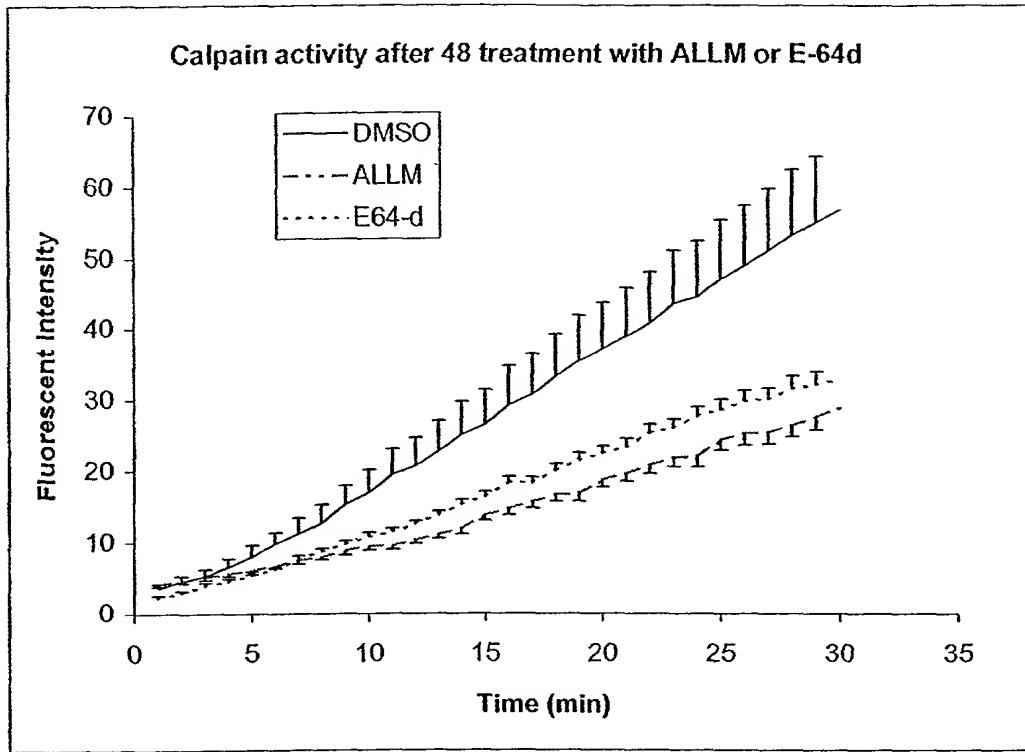


FIG. 26